

Effect of Hemodilution on Coagulation and Recombinant Factor VIIa Efficacy in Human Blood In Vitro

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Background: This study evaluates the effect of hemodilution by various common resuscitation fluids, and the efficacy of activated recombinant factor VII (rFVIIa) on coagulation parameters in human blood in vitro.

Methods: Samples from normal healthy volunteers ($n = 9$) were hemodiluted from 0% to 90% with normal saline, or 0%, 40%, 60%, and 80% with 5% albumin, Hespan, Hextend, normal saline, or lactated Ringer's, and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 30 minutes with and without rFVIIa ($1.26 \mu\text{g/mL}$).

Results: There was a strong correlation between the dilution of hemoglobin (Hb), platelets, or fibrinogen and coagulation parameters. Hemodilution 0% to 90% changed coagulation parameters (prothrombin time [PT], activated partial thromboplastin time [aPTT], and thromboelastography) in an exponential fashion; the greatest changes occurred after hemodilution lowered Hb $<6 \text{ mg/dL}$, platelet count $<100,000/\text{mm}^3$, and fibrinogen concentration $<200 \text{ mg/dL}$. PT and aPTT were significantly prolonged after 60% and 80% dilution for all fluids. Hemodilution of 60% and 80% significantly decreased clot strength (maximum amplitude) and the kinetics of clot development (α angle) and increased the clot formation time (K). Hemodilution with Hextend and Hespan decreased maximum amplitude and α angle $>5\%$ albumin, lactated Ringer's, or normal saline. rFVIIa significantly improved PT at 60% and 80% dilutions, and aPTT at 80% dilution. There was a significant effect of dilution, but not fluid type, on the efficacy of rFVIIa to change PT and aPTT, and the onset of clotting (R).

Conclusions: We have strong in vitro evidence that Hb $<6 \text{ mg/dL}$, platelet count $<100,000/\text{mm}^3$, and fibrinogen concentration $<200 \text{ mg/dL}$ can be used as indexes of hemodilution-induced coagulopathy. This study also shows that Hextend and Hespan tend to decrease the clotting ability $>5\%$ albumin or the crystalloids. rFVIIa significantly decreased PT at all dilutions and aPTT at the highest dilution. The effectiveness of rFVIIa on PT and aPTT was significantly affected by the degree of dilution, but not by the type of fluid.

Key Words: Hemorrhage, Coagulopathy, Hemodilution, Hemostasis, PT, aPTT, TEG.

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Hemorrhage and trauma are a major cause of death in young civilians and are the principal cause of death on the battlefield.^{1–3} In the United States, between the years 1997 and 2008, acute hemorrhage accounted for 30% of the overall mortality in trauma patients.³ Hemorrhagic shock and trauma followed by fluid resuscitation can lead to the development of coagulopathies that can complicate surgical attempts to stop the bleeding.^{1,4,5} The inability to clot can be caused by acidosis, hypothermia, and dilution of coagulating factors; all associated with hemorrhage and trauma.^{4,6,7} Of these three, hemodilution, resulting from after routine resuscitation with large volumes of crystalloid or colloid in an attempt to restore blood volume and blood pressure, is a problem that is often caused by the medical teams treating these patients. Hemodilution occurs. Currently, the Advanced Trauma Life Support protocol recommends resuscitation with 2 L of lactated Ringer's followed by packed red blood cells if the crystalloid fails to reverse the signs of shock.^{8,9} The military edition of the Prehospital Trauma Life Support recommends 1 L to 2 L of lactated Ringer's after hemorrhage is under control.¹⁰ Replacement of lost blood with 1 L to 2 L of nonplasma fluid represents (at least) a 30% to 40% dilution of blood volume in a 70-kg person,¹¹ which could lead to dilution of clotting factors and a decrease in coagulation.

Activated recombinant factor VIIa (rFVIIa) is a hemostatic agent approved for use in the treatment of hemophilia A and B with inhibitors, acquired hemophilia, congenital factor VII deficiency, and Glanzmann thrombasthenia.¹² In trauma, when a blood vessel is injured, tissue factor on subendothelial pericytes is exposed and binds to endogenous FVII, resulting in activation of FX. FXa complexes with FV and converts prothrombin to thrombin. Platelets, required for primary hemostasis, will complex to the exposed collagen and von Willebrand factor at the injured endothelial surface, providing both a thrombogenic surface to amplify the coagulation cascade and becoming structurally involved in the clot. Normally, endogenous anticoagulants circulate at high enough levels to neutralize Factor Xa when it is complexed by FVIIa/Tissue Factor and prevents inappropriate coagulation. However, injury to a vessel causes a local exposure of Tissue Factor at high enough concentrations to overcome the anticoagulants, and coagulation occurs.^{13–16} Over the past several years, rFVIIa has been used as a novel hemostatic agent for treating severe bleeding associated with traumatic hemorrhage.^{17–19} rFVIIa stimulates the activation of thrombin

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generation on the platelet surface and results in the formation of a hemostatic plug at the site of injury.^{20,21}

The development of dilutional coagulopathy seems to depend on the type of resuscitation fluid and the degree of hemodilution.^{22–24} Therefore, it would be beneficial for the trauma patient if the physician can identify a resuscitation fluid that would improve, or at least not worsen coagulation function as resuscitation stabilizes cardiovascular parameters. Furthermore, it would be beneficial to characterize the affect of coagulation factors like rFVIIa in hemodiluted conditions. Currently, there are various crystalloid and colloid solutions available for resuscitation, but their effects on coagulation are not fully elucidated. The hemostatic consequences of hemodilution have been the focus of several studies, with inconsistent results.^{23–26} Studies have demonstrated that hemodilution resulting from blood loss and fluid administration

can result in either a hypo- or hypercoagulation.^{25,26} The objectives of this in vitro study were to determine (1) how hemodilution with different fluids affects coagulation, (2) if hemodilution modulates the effectiveness of rFVIIa, and (3) if the activity of rFVIIa is dependent on the type of resuscitation fluid.

PATIENTS AND METHODS

Blood Collection

This study was conducted under a protocol reviewed and approved by the Brooke Army Medical Center Institutional Review Board. Subjects were screened for any known coagulation disorders, any drug prescription, or over-the-counter medication or supplement that could have an effect on coagulation function, and pregnancy. Any subject meeting any of these exclusion conditions was not included in this study. After informed consent was acquired, blood specimens were collected from nine normal healthy volunteers via venipuncture into sodium citrate vacutainers according to standard clinical operating procedures.

Coagulation

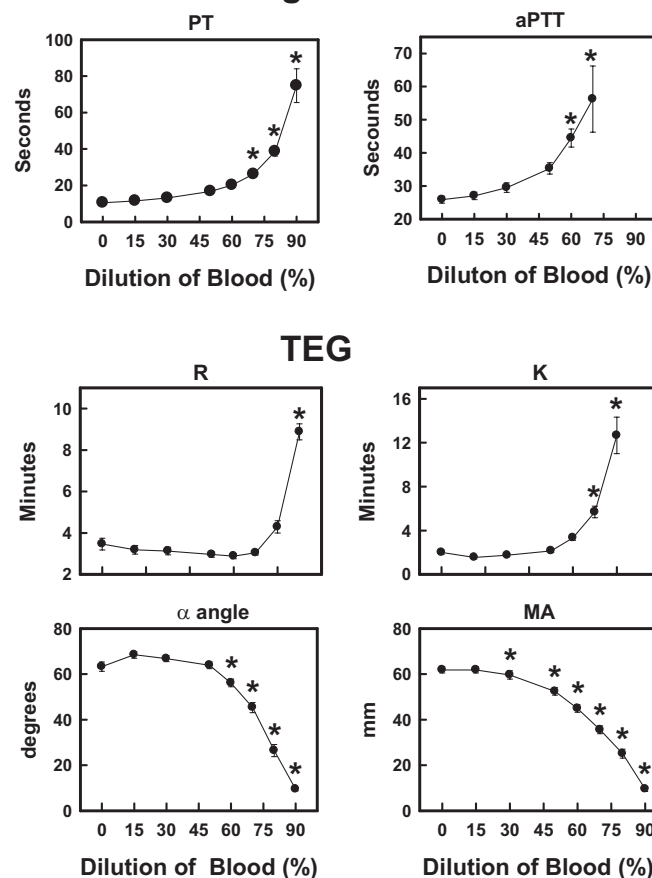


Figure 2. Coagulation parameters PT, aPTT, and TEG parameters R (time for initial fibrin formation), K (time of clot formation), α angle (kinetics of clot development), and MA (maximum clot strength). Values represent mean \pm standard error of the mean. * $p < 0.05$ compared with 0 dilution ($n = 9$ subjects).

Blood Chemistry

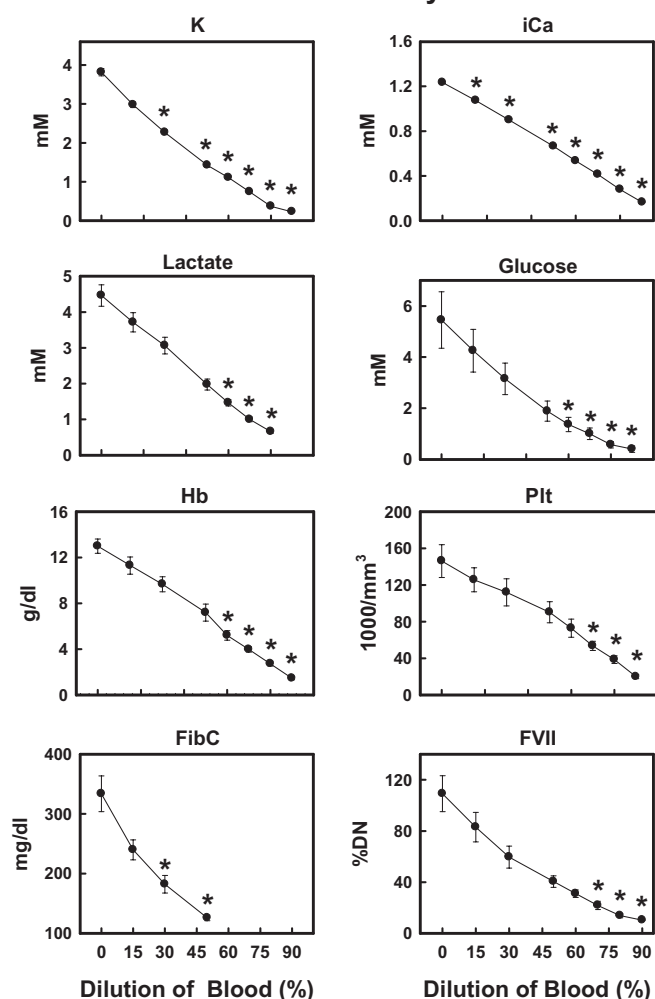


Figure 1. Plasma levels of K⁺, ionized Ca⁺⁺, lactate, glucose, hematocrit, platelet count, fibrinogen concentration, and factor VII in human blood diluted 0%, 15%, 30%, 50%, 60%, 70%, 80%, and 90% by normal saline and 20 mM HEPES. DN, Dade normal. Values represent mean \pm standard error of the mean. * $p < 0.05$ compared with 0 dilution ($n = 9$ subjects).

Hemodilution

Blood samples were incubated at 37°C for 30 minutes before experimentation. Initial experiments were performed with diluted blood ($n = 9$ subjects) at dilutions of 0% to 90% with normal saline and 20 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4). We found that blood chemistry varied directly with the degree of dilution (Fig. 1). However, hemodilution had a varied affect on coagulation (Fig. 2). After evaluation of these results, subsequent studies of hemodilution were performed at 0%, 40%, 60%, or 80% with 5% albumin (Baxter), Hespan (6% Hetastarch in 0.9% NaCl), Hextend (6% Hetastarch in a lactated Ringer's balanced electrolyte solution), lactated Ringer's, or normal saline, and incubated at $37^\circ \pm 1^\circ\text{C}$ for an additional 30 minutes. These fluids were selected for evaluation because they were Food and Drug Administration approved and available for use by US military medical personnel. Next, rFVIIa (NovoSeven, Novo Nordisk, Denmark) or the vehicle solution (saline) was added to the blood samples. The samples were then incubated for an additional 10 minutes and evaluated for coagulation function as described below. The final concentration of rFVIIa in blood was $1.26\text{ }\mu\text{g/mL}$, which is equivalent to a $90\text{-}\mu\text{g/kg}$ in vivo dose.

Hematological and Coagulation Tests

Blood samples ($n = 9$ subjects) were analyzed for blood chemistry, hematocrit, and platelet count using a

Pentra-120 hematology analyzer (ABX, Montpellier, France) according to the manufacturer's instructions. Blood samples were centrifuged, and platelet-poor plasma was collected for coagulation tests. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentration were measured in duplicate by standard clinical methods (BCS Coagulation Analyzer, Dade Behring, Deerfield, IL).

Thromboelastography

Thromboelastography (TEG) monitors changes in the viscoelastic properties of blood as a clot is being formed. The parameters that were measured included the reaction time (R , min, the time when the initial fibrin formation is detected); clotting time (K , min, the time of clot formation and is the time from the R time until a clot with a fixed firmness is formed); α angle (degree, the kinetics of clot development); and maximum amplitude (MA, mm, the maximum strength or firmness of the developed clot). All samples were run in triplicate on a Hemoscope Model 5000 (Skokie, IL) at 37°C and calibrated daily. Disposable cups and pins were loaded and allowed to equilibrate to temperature. Next, $10\text{ }\mu\text{L}$ tissue factor (Innovin, diluted 1:500), $20\text{ }\mu\text{L}$ of 0.2 mol/L CaCl_2 , and $4.3\text{ }\mu\text{L}$ of $19.2\text{ }\mu\text{g/mL}$ Corn Trypsin Inhibitor were added to each cup and allowed to equilibrate. Citrated blood ($340\text{ }\mu\text{L}$) was then added to each cup, and the TEGs were started

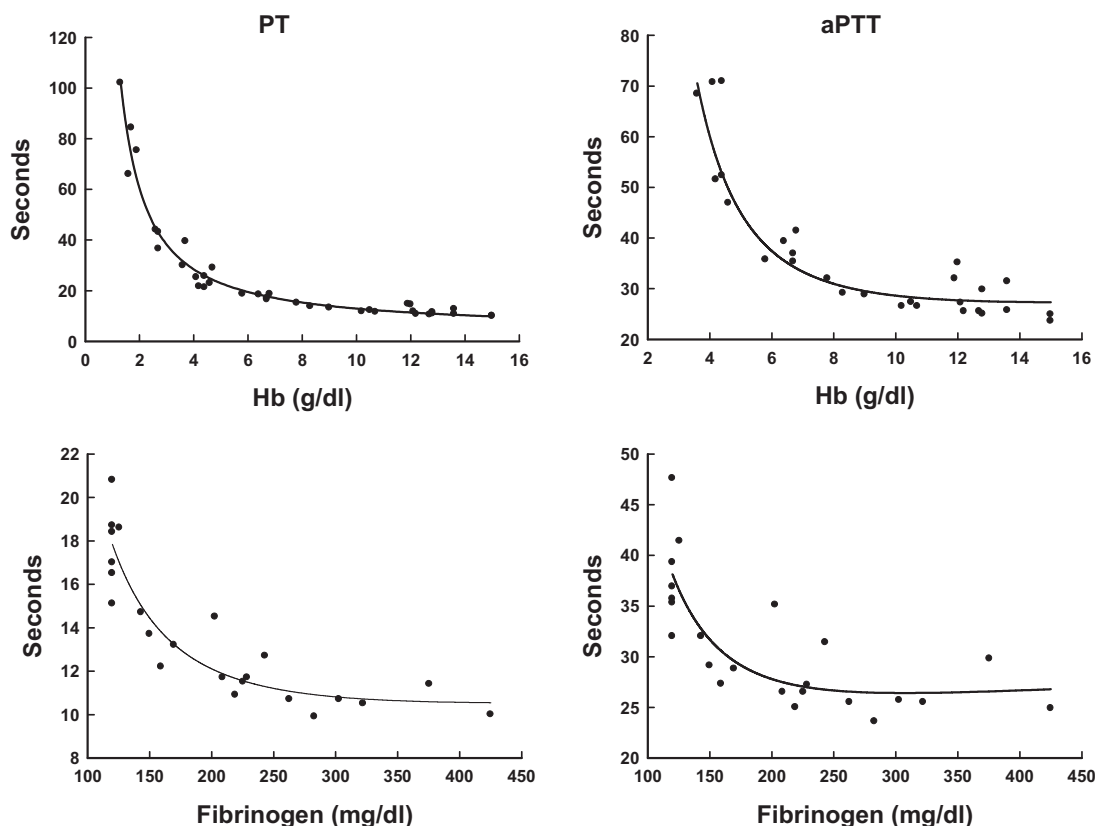


Figure 3. Correlation between total Hb (tHb) vs. PT and aPTT, and between fibrinogen concentration vs. PT and aPTT. Data were fitted to a nonlinear regression, second-order inverse polynomial $y = a + b/x + c/x^2$. Correlation coefficient and equation parameters are in Table 1 ($n = 9$ subjects).

immediately. The tests were terminated 30 minutes after MA was reached.

Data Analysis

Data were analyzed with one- or two-way analysis of variance (ANOVA; using dilution and solution type as the major variables) followed by multiple comparison analysis using the Holm-Sidak Method. Kruskal-Wallis one-way ANOVA on ranks was used if normality test on variance failed followed by Dunn's Multiple Comparison. Normality of population was tested by the Kolmogorov-Smirnow test. Natural log transform was applied to the change in PT and aPTT before two-way ANOVA. Our sample size of nine had sufficient power at 0.8 to detect a specified effect with $1 - \alpha$ confidence ($\alpha = 0.05$). Statistical analyses were performed by SigmaStat. $p < 0.05$ was considered significant. The data are expressed graphically and in tables as mean \pm standard error of the mean. Nonlinear regressions were performed by SigmaPlot. The significance of each regression was tested by ANOVA.

RESULTS

Our subject population ($n = 9$) showed normal values for hematocrit, PT, aPTT, and fibrinogen in undiluted blood. The mean values were $37.4\% \pm 1.0\%$, $10.3 \text{ s} \pm 0.1 \text{ s}$, $29.9 \text{ s} \pm 0.7 \text{ s}$, and $286 \text{ mg/dL} \pm 14 \text{ mg/dL}$, respectively. Platelets were $134 \times 10^3/\text{mm}^3 \pm 8 \times 10^3/\text{mm}^3$ for our subject population.

In the initial experiments, dilution of human blood from 0% to 90% with normal saline and 20 mmol/L HEPES (pH 7.4) showed a statistically significant decrease in various blood parameters that was proportional to the degree of dilution (Fig. 1). The dilution of blood had variable effects on coagulation parameters (Fig. 2). Both PT and aPTT increased exponentially after 60% hemodilution. However, the effects were not significant until 70%, 80%, and 90% dilution for PT, and 80% and 90% dilution for aPTT (Fig. 2). The TEG parameters R , K , α angle, and MA also changed exponentially. Although the effects were significant only at the highest dilutions for R and K , α angle decreased significantly at dilutions $\geq 60\%$, and MA decreased significantly at dilutions $\geq 30\%$ (Fig. 2). It is interesting to note that at the lower dilutions, R and K decreased, and α angle and MA slightly increased, although not significantly. These data suggest that higher dilutions resulted in hypocoagulation, whereas lower dilutions lead to some degree of hypercoagulation.

Dilution of blood showed a strong correlation between various coagulation parameters and hemoglobin (Hb), platelet count, and fibrinogen concentration (Figs. 3–6). The correlations tended toward two graphical relationships: an exponential-type increase (for PT, aPTT, R , and K) or a decrease (α angle and MA) with dilution. We used a second-order inverse polynomial to fit the exponential-type increase because it was the simplest of the nonlinear regressions that

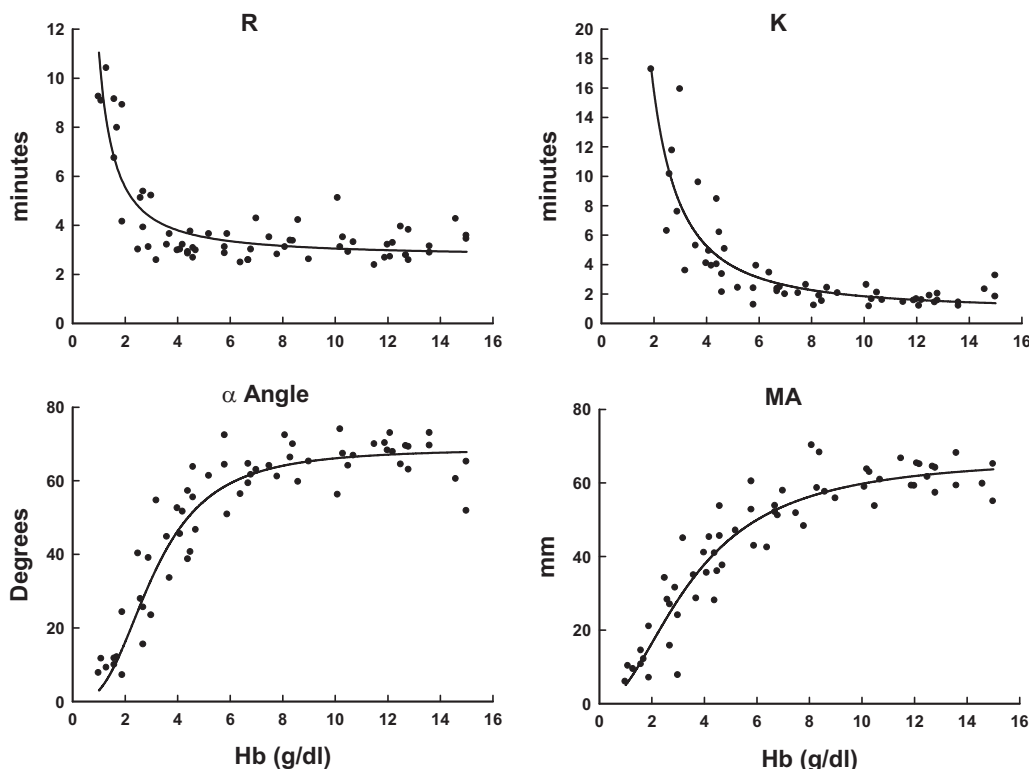


Figure 4. Correlation between tHb vs. TEG parameters, R , K , α angle, and MA. Data for R and K were fitted to a nonlinear regression, second-order inverse polynomial $y = a + b/x + c/x^2$. Data for α angle and MA were fitted to a three-parameter logistic equation $y = a/(1 + (x/c)^b)$. Correlation coefficient and equation parameters are in Table 2 ($n = 9$ subjects).

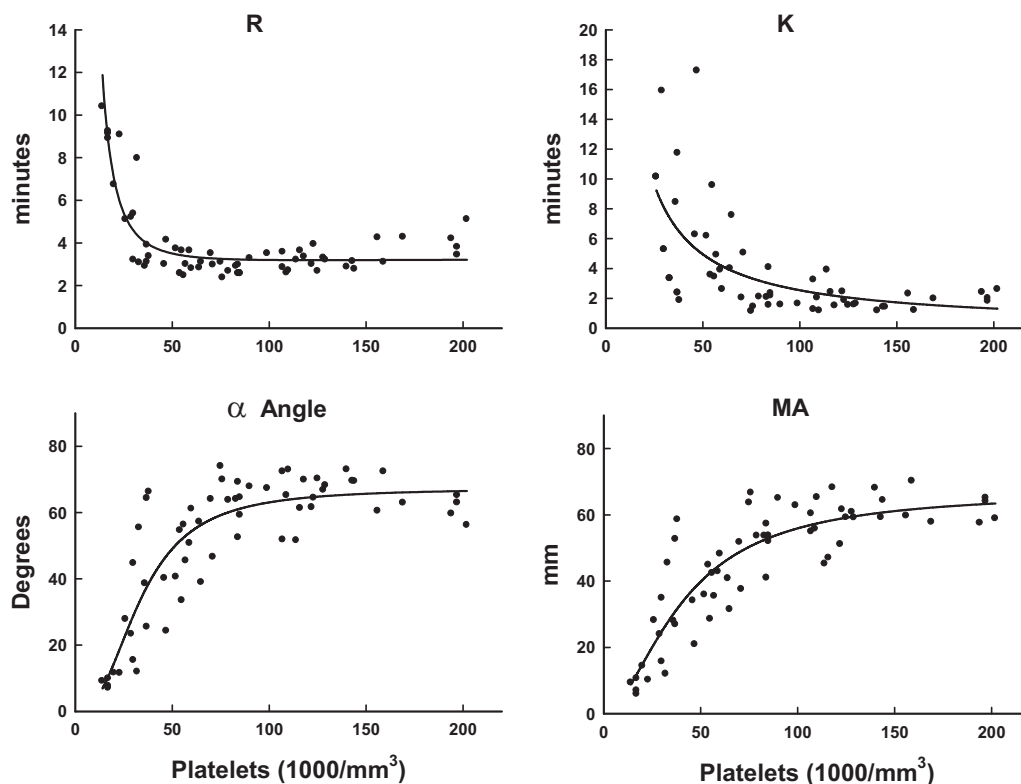


Figure 5. Correlation between platelet count vs. TEG parameters, R , K , α angle, and MA. Data for R and K were fitted to a nonlinear regression, second-order inverse polynomial $y = a + b/x + c/x^2$. Data for α angle and MA were fitted to a three-parameter logistic equation $y = a/(1 + (x/c)^b)$. Correlation coefficient and equation parameters are in Table 2 ($n = 9$ subjects).

gave the highest correlation coefficient. The decrease was fitted by a three-parameter logistic function for the same reasons. The equations used and parameters calculated are given in Tables 1 and 2. All regressions are significant ($p < 0.05$). Dilution of Hb and fibrinogen showed a strong correlation with the changes in PT and aPTT (Fig. 3, Table 1). The greatest changes in PT and aPTT were at Hb below 4 g/dL to 6 g/dL and at fibrinogen concentrations below 200 mg/dL. Dilution of Hb and platelet count also showed strong correlations with all TEG parameters (Figs. 4 and 5, Table 2). The greatest changes in TEG parameters were also at Hb below 4 g/dL to 6 g/dL and at platelet counts below 60 to 80,000/ mm^3 . R time was least affected by dilution and did not change until Hb was below 2 g/dL to 3 g/dL. The correlations between fibrinogen concentration and TEG parameters were not as strong (Fig. 6, Table 2). Based on these data, further experiments using dilutions of 0%, 40%, 60%, and 80% were designed to test five different commonly used resuscitation fluids (described below).

Hemodilution With 5% Albumin, Hespan, Hextend, Normal Saline, and Lactated Ringer's

Platelet count, fibrinogen concentration, and Hb decreased significantly ($p < 0.05$) in direct proportion to the degree of hemodilution with all fluids tested (Fig. 7). There was no significant difference among the fluid groups in the dilution of platelets, fibrinogen, or Hb (Fig. 7).

PT and aPTT

Hemodilution of human blood with all five fluid types led to a change in coagulation that varied directly with the amount of dilution. Furthermore, the effect of hemodilution on coagulation parameters was similar to dilution with normal saline and 20 mmol/L HEPES (Figs. 1 and 2). PT and aPTT became prolonged with hemodilution in all five fluid groups (Fig. 8). However, the increase was not significant until dilutions reached 60% and 80%. A significant difference between fluid types was observed by two-way ANOVA for both PT and aPTT. Post hoc analysis showed that PT was prolonged more in the 5% albumin and normal saline when compared with both Hespan and Hextend. Moreover, aPTT was prolonged more in the normal saline group when compared with both lactated Ringer's and Hextend.

Thromboelastography

Hemodilution led to significant effects in R , K , α angle, and MA with increasing dilutions of all the fluids (Fig. 9). Two-way ANOVA of R , K , α angle, and MA showed significant differences within fluid groups and significant differences within dilutions. R tended to shorten at 40% dilution in all groups, then increase. However, these changes were not significant after post hoc analysis of the two-way ANOVA. R was significantly longer after dilution to 60% and 80% with 5% albumin or lactated Ringer's, or 80% with Hespan or

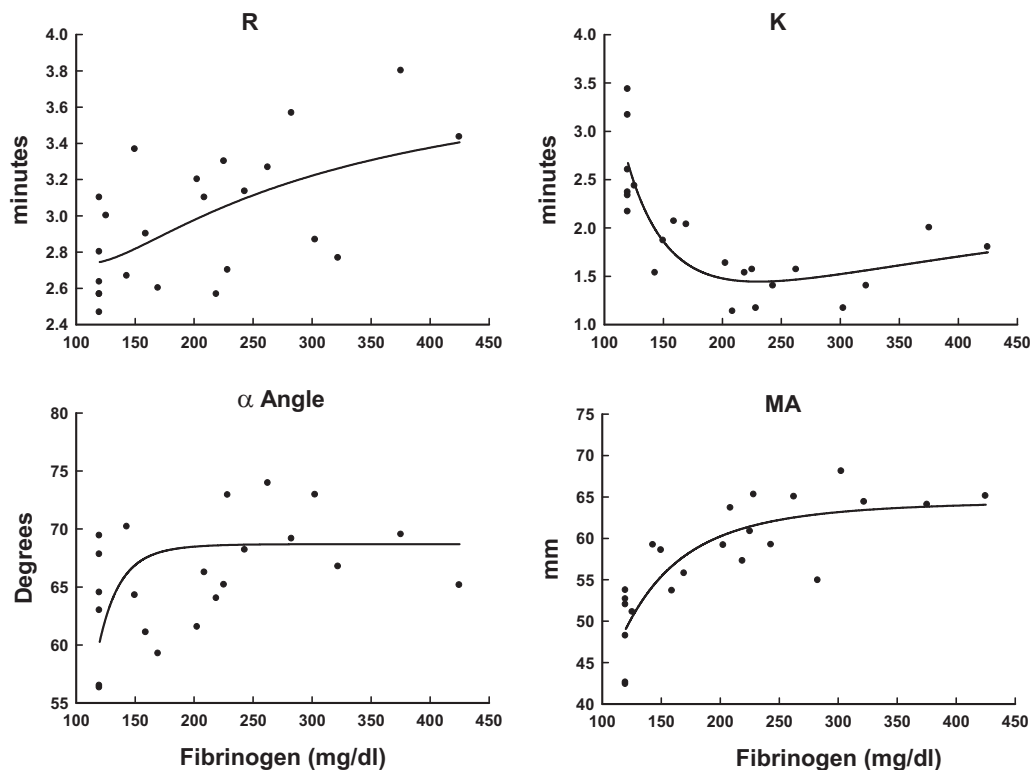


Figure 6. Correlation between fibrinogen concentration vs. TEG parameters, R , K , α angle, and MA. Data for R and K were fitted to a nonlinear regression, second-order inverse polynomial $y = a + b/x + c/x^2$. Data for α angle and MA were fitted to a three parameter logistic equation $y = a/(1 + (x/c)^b)$. Correlation coefficient and equation parameters are in Table 2 ($n = 9$ subjects).

TABLE 1. Nonlinear Regression Coefficients for Figure 3, Second-Order Polynomial $y = a + b/x + c/x^2$

	PT	aPTT
Hb vs.		
r^2	0.96	0.71
a	4.12	30.9
b	82.0	116
c	60.7	931
FibC vs.		
r^2	0.84	0.65
a	11.4	31.2
b	-800	-2,958
c	18,900	45,430

Hextend. Normal saline had no effect on R at any dilution. K was significantly longer after dilution with 40%, 60%, and 80% of Hespan, dilution with 60% and 80% of either 5% albumin or Hextend, or dilution with 80% of normal saline or lactated Ringer's. The α angle was significantly less after dilution with 40%, 60%, or 80% of Hespan or Hextend. Dilution with 5% albumin, normal saline, or lactated Ringer's significantly decreased α angle only at 80%. MA was significantly lower after dilution with 40%, 60%, and 80% of 5% albumin, Hespan, Hextend, or normal saline. Dilution with lactated Ringer's significantly decreased MA at 60% and 80%.

TABLE 2. Nonlinear Regression Coefficients for Figures 4–6

	$y = a + b/x + c/x^2$		$y = a/(1 + (x/c)^b)$	
	R	K	α Angle	MA
Hb vs.				
r^2	0.73	0.76	0.87	0.89
a	2.70	76.3	68.7	67.3
b	3.16	6.01	-2.75	1.99
c	5.24	47.9	3.07	3.52
Platelets vs.				
r^2	0.78	0.40	0.76	0.77
a	3.33	74.1	67.3	67.0
b	-34.4	24.9	-2.48	-1.78
c	2,154	-291	33.6	40.3
Fibrinogen vs.				
r^2	0.34	0.71	0.57	0.71
a	3.97	289	68.7	64.6
b	-275	-667	-7.34	-2.90
c	15,470	76,900	91.8	80.7

Correlation of Hb, platelets, or fibrinogen vs. TEG parameters to hemodilution.
Second-order inverse polynomial ($y = a + b/x + c/x^2$).
Three-parameter logistic equations ($y = a/(1 + (x/c)^b)$).

Within fluid groups, the responses of K , α angle, and MA were not different between Hespan and Hextend (Fig. 9). There was also no difference between 5% albumin, lactated Ringer's, and normal saline. However, there were significant

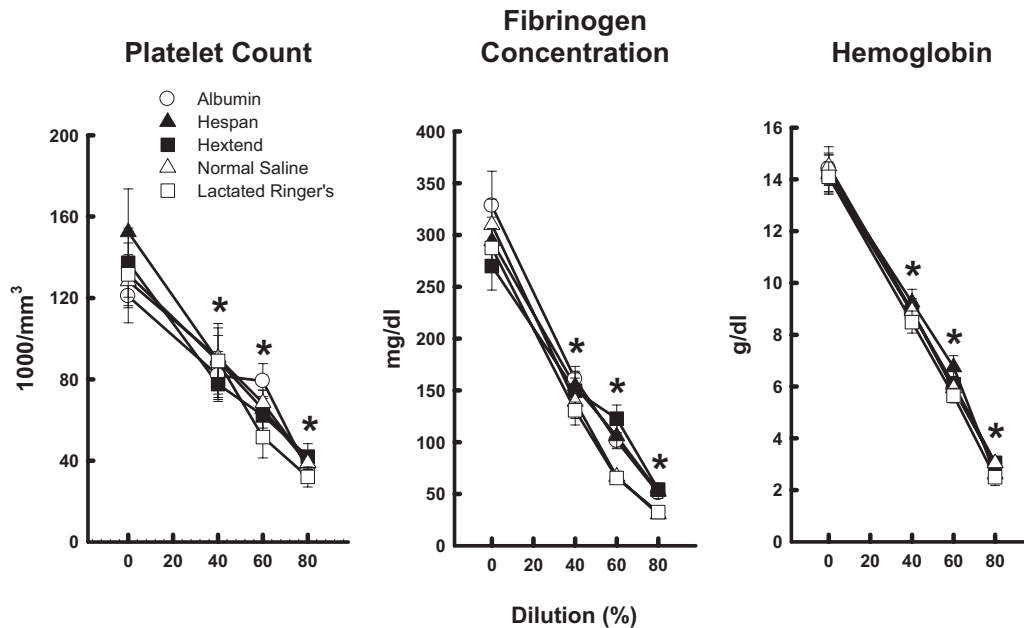


Figure 7. Platelet count, fibrinogen concentration, and hematocrit in human blood diluted 40%, 60%, and 80% by 5% albumin, Hespan, Hextend, normal saline, or lactated Ringer's. Values represent mean \pm standard error of the mean. * $p < 0.05$ from 0 dilution for all groups ($n = 9$ subjects).

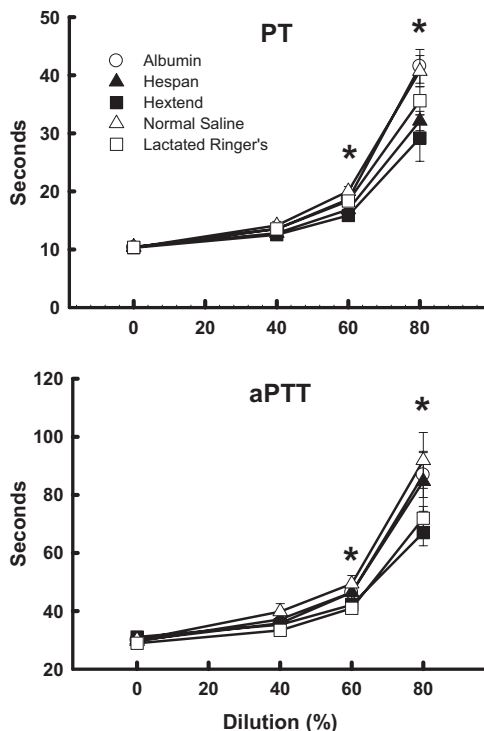


Figure 8. PT and aPTT in human blood diluted 40%, 60%, and 80% by 5% albumin, Hespan, Hextend, normal saline, or lactated Ringer's. Values represent mean \pm standard error of the mean. * $p < 0.05$ from 0 dilution in PT and aPTT for all groups ($n = 9$ subjects).

differences between Hespan or Hextend and the other three groups for K , α angle, and MA. Hespan and Hextend cause a greater hypocoagulation than the crystalloids or 5% albumin.

Effect of Hemodilution on rFVIIa Efficacy

Hemodilution significantly changed the efficacy of rFVIIa for both PT and aPTT in all groups (Table 3). rFVIIa significantly shortened PT after 60% and 80% dilution for all five fluids and after 40% dilution with albumin, Hespan, and lactated Ringer's (Table 3). rFVIIa significantly shortened aPTT after 80% dilution for all five fluids and after 60% dilution with normal saline (Table 3). We also looked at the change in PT and aPTT as defined as the difference before and after rFVIIa. We found a significant effect of dilution on the change in both PT and aPTT to rFVIIa by two-way ANOVA (Fig. 10). Furthermore, the effect varied in proportion to the degree of dilution.

rFVIIa significantly shortened R over all dilutions for Hextend and lactated Ringer's, 0%, 40%, and 60% for normal saline, 0% and 80% for albumin, and 0% for Hespan (Table 4). rFVIIa had little effect on the other TEG parameters.

Effect of Fluid Type on rFVIIa Efficacy

As expected, rFVIIa primarily affected PT and R time in the TEG, but there were no significant differences among the fluid types. This suggests that the activity of rFVIIa is not significantly affected by fluid type.

DISCUSSION

Our study looked at the effect of 0% to 90% hemodilution on coagulation parameters. We found a strong correlation between the dilution of Hb, platelets, and fibrinogen

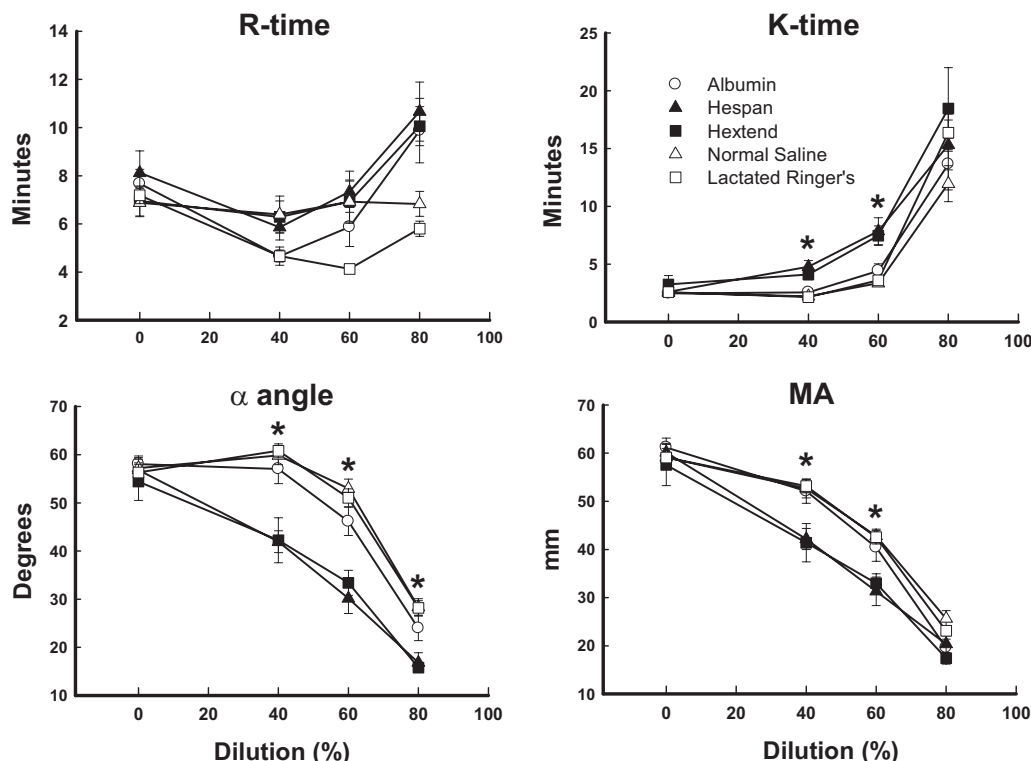


Figure 9. TEG parameters *R* (time to initial fibrin); *K* (speed of clot formation); α angle (kinetics of clot development); and MA (strength of clot) in human blood diluted 40%, 60%, and 80% by 5% albumin, Hespan, Hextend, normal saline, or lactated Ringer's. Values represent mean \pm standard error of the mean. * $p < 0.05$ differences between Hespan/Hextend and the other three groups ($n = 9$ subjects).

and coagulation parameters, and the greatest changes occurred at the higher dilutions (Figs. 2, 8, and 9). Although blood chemistry, fibrinogen concentration, and FVII levels fell in a linear fashion to progressive hemodilution, most of the coagulation parameters (PT, aPTT, and TEG) changed in an exponential fashion suggesting that dilution has a more complex effect on coagulation than simply dilution of any single coagulation factor like FVII or fibrinogen (Fig. 1). It is interesting to note that the exponential change was seen in the cellular (TEG) and cell-free coagulation measurement systems (PT and aPTT) suggesting that hemodilution may be affecting thrombin function. This exponential relationship is also seen with the correlation of Hb, fibrinogen concentration, or platelets with PT, aPTT, and TEG parameters (Figs. 3–6). Because the greatest changes in PT, aPTT, and TEG were at Hb below 4 g/dL to 6 g/dL, Hb may be an index that can be used to predict the degree of coagulopathy in a patient who has received nonplasma resuscitation fluids. Previous clinical studies have shown that Hb levels can be used to determine when the transfusion of red blood cells is needed in critical care patients.²⁷ Various standards have been suggested using Hb levels, platelet count, or fibrinogen concentration as “triggers” for transfusion of red blood cells, platelets, cryoprecipitate, plasma, or whole blood.^{28–32} Values that have been agreed on and are commonly used include Hb < 6 g/dL, platelet count $\leq 100,000/\text{mm}^3$, and fibrinogen concentration < 100 mg/dL. However, these values seem to be based more

on expert opinion than on research-based evidence. Our study clearly shows a strong correlation between Hb, platelets, and fibrinogen versus coagulation parameters during hemodilution that agree, for the most part, with these trigger values. Significant changes in PT, aPTT, *R*, *K*, α angle, and MA do not occur until Hb < 6 mg/dL, platelet count $< 100,000/\text{mm}^3$, and fibrinogen concentration < 200 mg/dL (Figs. 3–6). Weiss et al.³³ reported results suggesting an exponential change in aPTT during serial dilution to saline or hydroxyethyl starch (HES) up to 80% but a linear response of PT. In another study, Thygesen et al.³⁴ showed that serial dilution with HES up to 50% in pigs had little to no effect on PT and aPTT, which agrees with our findings.

We found that 40%, 60%, and 80% dilutions of human blood prolonged PT, aPTT, and *K*, and reduced MA and α angle, irrespective of what fluid was used. This finding is consistent with results from other laboratories. In vitro dilution of whole human blood with HES 130, HES 200, HES 600, HES 670, 5% albumin, lactated Ringer's, or NaCl was shown to significantly decrease MA and prolong *R* of TEG.^{22,35} Hemodilution with saline or lactated Ringer's prolonged clotting time and reduced maximum clot firmness as measured by Rotational Thrombelastogram.^{36–38} Hemodilution of $\sim 50\%$ or 75% with Hextend, Dextran-70, 5% albumin, Pentalyte, or HES in rabbits prolonged PT and aPTT and decreased MA and α angle.^{23,39} In pigs, hemodilution up to 70% with HES 130 or HES 650 significantly prolonged PT

TABLE 3. Effect of rFVIIa on PT and aPTT

rFVIIa	PT (s)		aPTT (s)	
	Before	After	Before	After
Albumin				
0%	10.3 ± 0.4	8.8 ± 0.3	29.8 ± 2.1	26.4 ± 1.7
40%	13.6 ± 0.6	9.8 ± 0.2*	35.8 ± 1.7	31.7 ± 1.6
60%	18.5 ± 1.0	12.1 ± 0.4*	46.4 ± 2.6	39.8 ± 2.5
80%	41.5 ± 2.9	25.1 ± 1.4*	86.8 ± 7.7	70.0 ± 4.4*
Hespan				
0%	10.5 ± 0.3	8.8 ± 0.3	29.6 ± 2.2	27.1 ± 0.9
40%	12.8 ± 0.7	9.9 ± 0.4*	37.1 ± 2.3	33.0 ± 1.9
60%	16.9 ± 0.8	11.7 ± 0.9*	46.1 ± 2.2	41.3 ± 3.5
80%	32.1 ± 1.7	19.1 ± 1.1*	84.7 ± 10.3	70.1 ± 9.4*
Hextend				
0%	10.3 ± 0.2	8.9 ± 0.9	31.1 ± 1.4	29.9 ± 2.8
40%	12.5 ± 0.6	9.9 ± 0.4	35.3 ± 1.8	31.5 ± 2.4
60%	15.8 ± 1.0	10.6 ± 0.8*	42.1 ± 2.9	35.9 ± 3.4
80%	29.2 ± 4.0	17.7 ± 2.1*	67.0 ± 4.5	52.5 ± 7.2*
Normal saline				
0%	10.3 ± 0.1	9.1 ± 0.7	30.0 ± 9.6	28.0 ± 2.0
40%	14.1 ± 0.7	10.8 ± 0.8	39.8 ± 2.8	32.7 ± 1.7
60%	20.0 ± 0.8	12.5 ± 0.6*	49.4 ± 2.9	39.1 ± 3.1*
80%	40.7 ± 2.7	27.8 ± 2.2*	91.9 ± 9.6	73.9 ± 5.1*
Lactated Ringer's				
0%	10.4 ± 0.3	8.6 ± 0.3	28.9 ± 0.8	25.9 ± 0.8
40%	13.5 ± 0.5	9.8 ± 0.1*	33.4 ± 1.1	29.2 ± 1.1
60%	18.3 ± 0.7	12.8 ± 0.3*	41.0 ± 1.7	37.1 ± 2.4
80%	35.6 ± 2.4	25.7 ± 1.2*	72.0 ± 4.1	62.2 ± 3.1*

Values represent mean ± standard error of the mean.

* $p < 0.05$ when compared with no rFVIIa.

and aPTT and decreased MA and α angle.³⁴ Our study showed that Hespan and Hextend tended to cause a greater hypocoagulation with increasing dilution when compared with the crystalloids or 5% albumin as defined by TEG (Fig. 9). However, the measurement of PT and aPTT did not show a greater hypocoagulation by Hespan and Hextend (Fig. 8). The mechanism by which hetastarch impairs coagulation has been extensively reviewed and involves decreasing circulating levels of factor VIII and von Willebrand factor, and impairs binding of soluble fibrinogen to platelet cell surface glycoproteins Ib-IX and IIb-IIIa, leading to less platelet rolling, adhesion, and activation.^{40,41}

Our data clearly show that hypocoagulation occurs after hemodilution 60% to 80%. However, a hypercoagulation is suggested at the lower dilutions based on *R* time with all fluids except normal saline (Figs. 2 and 9). This finding is consistent with reports from other groups^{25,26} showing a shorter *R* and reduced MA values at dilutions $\leq 50\%$, suggesting that the type of coagulopathy may change as hemodilution increases. A low level of hemodilution may lead to hypercoagulation. As dilution increases, the coagulopathy changes to hypocoagulation. The cause of this change is most likely because of the effect of diluting pro- and anticoagulant factors in blood. It is possible that low-level hemodilution

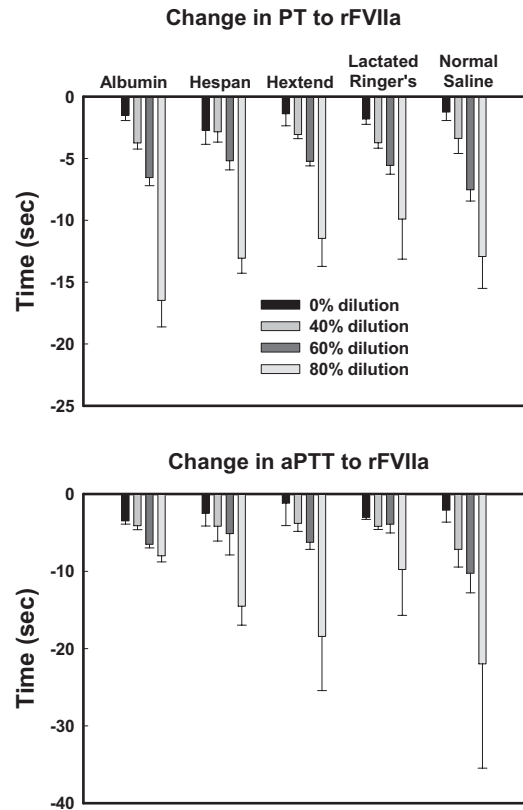


Figure 10. The change in PT and aPTT after addition of rFVIIa in human blood diluted 40%, 60%, and 80% by 5% albumin, Hespan, Hextend, normal saline, or lactated Ringer's. The change was calculated as after-before rFVIIa was added. Values represent mean ± standard error. There was significant effect of dilution on the change in both PT and aPTT to rFVIIa by two-way ANOVA ($n = 9$ subjects).

preferentially affects the anticoagulant factors leading to hypercoagulation. As the hemodilution increases, the procoagulant factors are diluted leading to hypocoagulation. In vitro studies have shown that hemodilution causes a significant decrease in coagulation factors such as fibrinogen, factors II, VII, IX, and X^{23,37,42} as well as the generation of thrombin.⁴³ Coagulopathies have been reported in severe trauma patients,⁶ and these have been partially attributed to hemodilution by resuscitating agents.^{4,7,44} Dilution of coagulation factors and administration of anticoagulants could partially explain the hypocoagulation seen in patients who have undergone massive transfusions.⁴⁵

A mathematical model of the coagulation cascade has been reported by Zhu.⁴⁶ Taking into account both intrinsic and extrinsic systems, the kinetics of each reaction, the presence of inhibitors, and feedback effects, the model predicts that increasing dilution of factors II, VII, IX, X, XI, and XII leads to an increase in clotting time that is exponential in fashion. The model also predicts that the changes in clotting time are greater after 40% to 50% dilution. This finding has important clinical effects as administration of 1 L to 2 L of colloids or crystalloids is routinely used to resuscitate hemorrhage/trauma patients.³⁰ A blood loss of 1 L to 2 L repre-

TABLE 4. TEG Parameters Derived From Diluted Blood Before and After rFVIIa

rFVIIa	R (min)		K (min)		α Angle (Degrees)		MA (mm)	
	Before	After	Before	After	Before	After	Before	After
Albumin								
0%	7.7 \pm 0.6	4.7 \pm 0.5*	2.5 \pm 0.2	1.6 \pm 0.1	58.1 \pm 1.7	66.7 \pm 1.2*	61.2 \pm 1.9	63.9 \pm 1.8
40%	4.7 \pm 0.4	4.0 \pm 0.5	2.6 \pm 0.3	2.3 \pm 0.2	57.0 \pm 3.0	59.0 \pm 2.1	52.1 \pm 2.5	52.7 \pm 2.2
60%	5.9 \pm 0.8	5.0 \pm 0.6	4.4 \pm 0.6	4.3 \pm 0.6	46.1 \pm 2.9	47.1 \pm 3.2	40.4 \pm 2.9	40.4 \pm 2.5
80%	10.0 \pm 1.3	7.5 \pm 0.8*	13.7 \pm 2.2	10.5 \pm 1.2*	24.0 \pm 2.6	25.4 \pm 2.4	19.6 \pm 1.8	20.5 \pm 1.7
Hespan								
0%	8.1 \pm 0.9	4.9 \pm 0.4*	2.6 \pm 0.2	1.8 \pm 0.1	56.8 \pm 2.2	64.8 \pm 1.8*	60.1 \pm 1.8	62.2 \pm 1.9
40%	5.9 \pm 0.5	4.4 \pm 0.3	4.8 \pm 0.5	4.1 \pm 0.5	41.9 \pm 2.3	45.8 \pm 5.6	42.2 \pm 2.2	44.2 \pm 2.1
60%	7.3 \pm 0.9	5.4 \pm 0.6	7.9 \pm 1.2	8.4 \pm 1.2	30.2 \pm 3.1	31.4 \pm 2.6	31.3 \pm 3.0	33.5 \pm 2.2
80%	10.7 \pm 1.2	9.1 \pm 0.8	15.3 \pm 2.2	21.6 \pm 1.4*	16.8 \pm 2.1	14.6 \pm 0.8	20.4 \pm 2.3	17.8 \pm 0.9
Hextend								
0%	7.0 \pm 0.6	4.9 \pm 0.5*	3.2 \pm 0.7	3.4 \pm 1.8	54.4 \pm 3.9	60.5 \pm 6.9	57.5 \pm 4.3	58.0 \pm 5.8
40%	6.3 \pm 0.6	4.0 \pm 0.3*	4.1 \pm 0.5	4.2 \pm 0.5	42.2 \pm 4.7	45.7 \pm 2.3	41.4 \pm 4.0	43.9 \pm 1.8
60%	6.9 \pm 0.9	4.8 \pm 0.5*	7.5 \pm 0.8	5.5 \pm 0.8	33.4 \pm 2.6	40.7 \pm 3.7	33.0 \pm 2.0	41.6 \pm 3.2
80%	10.1 \pm 0.8	6.9 \pm 0.9*	18.5 \pm 3.5	15.4 \pm 3.4	15.8 \pm 0.9	26.2 \pm 6.7	17.4 \pm 1.2	27.9 \pm 5.9
Normal saline								
0%	6.9 \pm 0.5	4.2 \pm 0.3*	2.5 \pm 0.2	1.8 \pm 0.2	57.2 \pm 2.2	65.4 \pm 2.1*	59.1 \pm 1.9	60.9 \pm 3.4
40%	6.4 \pm 0.9	4.5 \pm 0.5*	2.2 \pm 0.2	1.8 \pm 0.2	59.8 \pm 1.9	64.7 \pm 2.0	52.7 \pm 2.0	56.3 \pm 2.0
60%	6.9 \pm 0.9	4.8 \pm 0.5*	3.3 \pm 0.2	2.9 \pm 0.3	53.0 \pm 2.0	57.9 \pm 2.5	42.8 \pm 1.4	46.4 \pm 2.0
80%	6.8 \pm 0.5	6.0 \pm 0.4	12.0 \pm 1.6	13.8 \pm 1.3	28.3 \pm 1.8	31.1 \pm 2.9	25.7 \pm 1.6	24.6 \pm 1.3
Lactated Ringer's								
0%	7.2 \pm 0.4	4.5 \pm 0.2*	2.6 \pm 0.2	2.9 \pm 1.2	56.3 \pm 1.7	62.0 \pm 4.5	59.1 \pm 0.8	58.0 \pm 4.5
40%	4.7 \pm 0.2	3.2 \pm 0.2*	2.2 \pm 0.1	1.9 \pm 0.2	60.8 \pm 1.4	64.8 \pm 1.5	53.2 \pm 1.4	55.6 \pm 12.0
60%	4.1 \pm 0.2	3.3 \pm 0.4*	3.6 \pm 0.3	3.4 \pm 0.4	51.0 \pm 1.8	54.6 \pm 2.2	42.5 \pm 1.4	44.3 \pm 1.4
80%	5.8 \pm 0.3	4.5 \pm 0.3*	16.4 \pm 1.6	12.9 \pm 2.2	28.2 \pm 1.5	32.2 \pm 5.8	23.1 \pm 1.0	28.1 \pm 1.6

Values represent mean \pm standard error.* $p < 0.05$ as compared with no rFVIIa ($n = 9$).

sents 20% to 40% of blood volume from a 70-kg person.¹¹ Our data and the mathematical model of Zhu⁴⁶ suggest that dilutions of 40% and greater could lead to serious coagulopathies. Our in vitro results support the clinical practice of using not more than 1 L to 2 L of colloids as a prehospital resuscitation fluid as outlined by Prehospital Trauma Life Support and the Advanced Trauma Life Support,^{8,10} and exceeding this or coadministering crystalloid will likely push the system over the edge and cause hypocoagulation. This analysis also takes into account the fact that crystalloids only stay in the vascular space a short time, whereas colloids like Hextend and Hespan stay considerably longer.⁴⁷ However, caution must be used when extrapolating the results of in vitro studies into clinical use. Similar in vivo studies must be done measuring the changes in hemostasis during hemodilution.

This study clearly shows that the efficacy of rFVIIa is affected by the degree of dilution as shown by the change of PT and aPTT (Table 3 and Fig. 10). rFVIIa improved PT, and aPTT after progressive hemodilution increased clotting time (Table 3). The improvement of clotting time by rFVIIa has been reported by others.^{39,42} Our study found that rFVIIa had little effect on K, α angle, and MA, although R was significantly shortened with rFVIIa over all dilutions and fluid types (Table 4). This finding is similar to other in vitro studies with human blood^{35,37–38} showing that rFVIIa tends to shorten the

initial coagulation time (R), but had variable effects on K, α angle, and MA.

Currently, rFVIIa is being used off-label for the treatment of severe nonsurgical bleeding. Data have become available from randomized, blinded studies on the use of rFVIIa for major liver resection,⁴⁸ prostatectomy,⁴⁹ and cirrhosis-induced gastrointestinal bleeding.⁵⁰ rFVIIa has been used off-label in an attempt to reduce mortality caused by diffuse and/or excessive bleeding in trauma patients and has been suggested to reduce 30-day mortality in severely injured combat casualties¹⁹ and decrease the need for packed red blood cell use in 20% of trauma patients requiring massive transfusion.⁵¹ In randomized, placebo-controlled, double-blind clinical trials, the use of rFVIIa resulted in significantly less red blood cell transfused in blunt trauma patients.⁵² The beneficial effects of rFVIIa have been reported in case studies and include diminished bleeding, improved visibility in the surgical field, and a decreased need for blood products.^{53–55} However, a recent report by Wade et al.⁵⁶ questions these conclusions and suggests that the undetected bias of patient selection may account for some of the reported beneficial effects of rFVIIa. Furthermore, Hauser et al.⁵⁷ demonstrated in a phase 3 randomized clinical trial that rFVIIa did not reduce mortality to major trauma (when compared with placebo), although it did reduce the need for blood products.

This suggests that a distinction exists between the hemostatic effect of rFVIIa and any potential influence on survival. It is possible that rFVIIa would be more beneficial for survival to trauma and hemorrhage when used in combination with other procoagulants. A recent study showed that increasing the dose of prothrombin complex concentrate increases thrombin generation to rFVIIa stimulation.^{58,59} This suggests that the addition of prothrombin complex concentrate before the use of rFVIIa clinically may be far more beneficial to hemostasis than rFVIIa alone. However, the safety of such a combination needs further study before it should be used in trauma patients.

Limitations

The limitations of this study relate to its in vitro nature, and the observations that the majority of the changes may occur at dilutions beyond those expected in surviving patients. However, such levels may help define resuscitation cut-off points for the various fluid types. In addition, the study only used one dose of rFVIIa. Although this dose was relevant to human clinical studies and our previous bleeding studies in pigs, it is possible that rFVIIa could have induced other changes if different doses were used to compensate for the dilution.⁵⁸ Moreover, it must be noted that the platelet counts in our subjects were low for normal (averaged between 125 and 155 × 1,000/mm³ in all groups). However, our in vitro experiments clearly showed that coagulopathies did not occur until platelet counts fell below 60 to 75 (Fig. 5).

CONCLUSION

We found that 0% to 90% hemodilution of whole blood (or plasma) changed coagulation parameters (PT, aPTT, and TEG) in an exponential fashion. The greatest changes in PT, aPTT, and TEG parameters occurred after hemodilution lowered Hb <6 mg/dL, platelet count <100,000/mm³, and fibrinogen concentration <200 mg/dL. However, it must be noted that Hb, platelet count, and fibrinogen concentration are markers of dilution, rather than a definitive cause of coagulation changes, and that the in vivo results will differ after red cell transfusion and release of factors V and VIII that can occur with trauma. We also found that serial dilution of human blood with five readily available resuscitation fluids leads to a hypocoagulation. Hespan and Hextend tended to cause a greater hypocoagulation (as measured by TEG but not by PT and aPTT) with greater dilution when compared with 5% albumin and the crystalloids. Furthermore, the efficacy of a therapeutic dose of rFVIIa was dependent on the degree of dilution, but not the fluid type, as measured by PT and aPTT.

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